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AVIAN REPELLENCY OF CONIFERYL AND CINNAMYL DERIVATIVES[1]

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Abstract. Phenylpropanoids, a class of common phenolic compounds in plants, may potentially be useful as pest repellents. We investigated the relationship between the chemical structure of coniferyl benzoate and its repellency to birds by comparing coniferyl benzoate to two analogous natural esters, corresponding alcohols, and benzoic acid. The absolute and relative feeding repellency of these compounds were assessed in choice (two-cup) and no-choice (one-cup) tests using European Starlings (*Sturnus vulgaris*). In addition, benzoin Siam (= gum benzoin Siam) was compared to coniferyl benzoate to ascertain if phenolics that naturally occur with coniferyl benzoate in benzoin Siam enhance its repellency.

Two-cup tests suggested that coniferyl alcohol was the most repellent compound followed by 3,4-dimethoxycinnamyl alcohol, 3,4-dimethoxycinnamyl benzoate, cinnamyl alcohol, cinnamyl benzoate, coniferyl benzoate, and benzoic acid. The repellency of most alcohols relative to their corresponding ester reversed in the one-cup tests. One-cup tests suggested that 3,4-dimethoxycinnamyl benzoate was the most repellent substance followed by cinnamyl benzoate, benzoin Siam, 3,4-dimethoxycinnamyl alcohol, cinnamyl alcohol, coniferyl alcohol, coniferyl benzoate, and benzoic acid.

Three conclusions on structure–activity relationships were inferred from these data. First, benzoate esters are more repellent than their corresponding alcohols. Second, repellency is increased by electron-donating groups. Third, acidic functions decrease repellency. We suggest that one function of naturally occurring coniferyl and cinnamyl derivatives may be chemical defense. Genetically engineering agricultural crops to produce analogs of coniferyl alcohol, as an inherent defense against pests and pathogens, may be possible.

Key words: allelochemical; avian pests; cinnamyl alcohol; coniferyl benzoate; feeding repellent; genetic engineering; phenols; phenylpropanoid; structure–activity relationships; Sturnus vulgaris.

INTRODUCTION

Natural products from higher plants and their analogs are an important source of new agrochemicals (Cardellina 1988, Cutler 1988). These natural chemicals often pose little environmental risk due to their low potential for hazardous bioaccumulation and their specific biological action (Cardellina 1988). Phenylpropanoids, a class of common phenolic compounds in plants (Robinson 1983), may potentially be useful as pest deterrents. In addition to their more familiar roles in plant metabolism, phenylpropanoids help protect plants in a number of ways. Phenylpropanoids can act as herbivore repellents, ultraviolet radiation shields, and phytoalexins ("antibodies"), and are structural components in lignin and suberin (Keen and Littlefield 1979, Hahlbrock and Scheel 1989, Lamb et al. 1989, Lynn and Chang 1990). Repellent phenylpropanoids are also utilized by various species of pharmacophagous insects, which produce these compounds as protection against vertebrate and invertebrate predators (Nishida and Fukami 1990).

Phenylpropanoids closely related to coniferyl alcohol and cinnamyl alcohol, in particular, may represent especially promising candidates for agrochemicals, because of their repellency to birds (Jakubas et al. 1989, Jakubas and Gullion 1990), insects (Cowles et al. 1990, Jakubas and Gullion 1991), and plant pathogens (Keen and Littlefield 1979). In addition, compounds related to cinnamyl alcohol are relatively nontoxic to humans

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(Hoskins 1984). Currently, there is a need to develop nonlethal repellents to control avian crop depredation and accidental bird poisonings (Bullard and York 1985, Dolbeer 1988, Mason et al. 1989, Crocker and Perry 1990). For example, wider use of nonlethal repellents could lessen the ecological impacts from the annual spraying of billions of Red-billed Quelea (*Quelea quelea*) with lethal avicides (see Bruggers et al. 1989).

The present study was designed to evaluate the relationship between the structure of coniferyl benzoate and its repellency (i.e., causing food avoidance) to birds. Coniferyl benzoate was chosen as the model compound because of its effect on the feeding behavior of wild birds (see Jakubas and Gullion 1991). Our experiments focused on the importance of phenyl ring substituents and the benzoate ester to the repellency of coniferyl benzoate. Two analogs of coniferyl benzoate, cinnamyl benzoate and 3,4-dimethoxycinnamyl benzoate, that occur in plants (Furia and Bellanca 1975, Kuroyanagi et al. 1985) were selected to study how electron-donating groups on the phenyl ring affect feeding repellency (Table 1). The importance of the benzoate ester was determined by comparing the feeding repellency of coniferyl benzoate and its two analog esters to their corresponding alcohols (Table 1). Additionally, benzoic acid was tested to determine the contribution of this moiety to feeding deterrency. The relative and absolute repellency of each compound was evaluated using choice and no-choice tests.

Methods

Chemical synthesis

A Bruker AM-500, 5 mm probe, nuclear magnetic resonance (NMR) was used to confirm all chemical syntheses.

Coniferyl benzoate was obtained by continuous liquid/liquid extraction of benzoin Siam tears number 3 (Alfred L. Wolff, Paris, France). Briefly, benzoin Siam tears were dissolved in a solution of methanol and water (90:10) (Shinobu Kato, Shiseido Laboratories, Yokohama, Japan, *personal communication*), filtered, and decanted into a large round-bottom flask. The filtered solution was extracted with pentane in a high volume Kontes (Vineland, New Jersey) liquid/liquid extraction system. During the extraction, the solution in the collection flask was magnetically stirred and not allowed to exceed 38°C. Extraction periods lasted 24–48 h and could be repeated up to 3 times per batch. Upon completion of the extraction, pentane was evaporated from the collected solution, and the crude coniferyl benzoate purified by crystallization (ether/pentane at −17°C for a minimum of 24 h [Daniel Joulain, Robertet, Grasse, France, *personal communication*]). Analysis of the crystallized product by proton NMR indicated that peak assignments matched those reported for coniferyl benzoate (Jakubas et al. 1989).

Coniferyl alcohol was synthesized using Lindeberg's (1980) procedure, with minor modifications. First, eugenol was acetylated with acetic anhydride in pyridine (solute ratio 1 mol:2 mol). Eugenol acetate thus obtained was brominated via N-bromosuccinimide (1 mol:1.15 mol) in carbontetrachloride with barium carbonate as an acid-scavenger (solute ratio 1 mol:1.5 mol). The bromo derivative thus obtained was dissolved in N,N-dimethylformamide. Potassium acetate (in molar ratio 10:1 potassium acetate : bromo derivative) was added and the mixture was kept at 90°C for 1 h. After evaporating the N,N-dimethylformamide under reduced pressure, the residue was dissolved in dichloromethane, washed with water, dried, and evaporated. The crude coniferyl diacetate was deesterified with lithium tetrahydroaluminate. The resulting product was purified by column chromatography (silica gel, hexane : ethyl acetate [2:1]) and crystallized to get coniferyl alcohol (melting point 72°–74°C observed; mp 72–73°C, Allen and Byers 1949).

Cinnamyl benzoate was synthesized by benzoylating cinnamyl alcohol (Aldrich Chemical Company, Milwaukee, Wisconsin). Briefly, benzoyl chloride was added to a mixture of cinnamyl alcohol in pyridine (5 mol cinnamyl alcohol:1 mol benzoyl chloride) at 0°C and left to warm to room temperature (≈ 23°C) overnight. When the reaction was complete, methylene chloride and water were added to the reaction mixture and the layers separated. The organic layer was washed with 1 mol/L hydrochloric acid, neutralized with a saturated solution of sodium bicarbonate, washed with water, dried, and evaporated using toluene and a rotary evaporator. The residue was purified by column chromatography (silica gel, hexane : ethyl acetate [10:1]) and crystallized.

3,4-dimethoxycinnamyl alcohol was synthesized from 3,4-dimethoxycinnamic acid following the procedure of Ponpipom et al. (1987). 3,4-dimethoxycinnamyl benzoate was synthesized by benzoylating 3,4-dimethoxycinnamyl alcohol using procedures identical to those used for cinnamyl benzoate. The product was purified by column chromatography (silica gel, hexane : ethyl acetate [2:1]), and crystallized in ether and pentane at −17°C. Melting points and proton NMR peak assignments of the synthesized products agreed with published data for 3,4-dimethoxycinnamyl alcohol and benzoate (see Kuroyanagi et al. 1985, Ponpipom et al. 1987).

Benzoic acid used in the feeding trials was obtained from Aldrich Chemical Company, Milwaukee, Wisconsin.

Diet preparation

Compounds to be tested were added to the birds' test feed (5:1 mixture of chick starter and AVN canary/finch diet [Purina Mills, Saint Louis, Missouri]) by dissolving the compounds in ethyl ether, mixing the ether solution with the feed, and then evaporating the ether under a ventilation hood. All diets were stored at −17°C in closed containers until they were presented the 2 wk before pretreatment, birds were provided free access to a 5:1 feed mixture of chick starter and AVN canary/finch diet (Purina Mills, Saint Louis, Missouri), and oyster shell grit (United Volunteer Aviaries, Nashville, Tennessee). Tap water was always available.

Each chemical was evaluated in one-cup (no-choice) and two-cup (choice) tests. In one-cup tests, intake of a test substance (i.e., treatment diet) during a given period is compared to the intake of another substance (i.e., control diet) presented at a different time. In two-cup tests animals are presented two diets and are able to simultaneously discriminate between them (Grote and Brown 1971). For our experiments, one- and two-cup tests were composed of a 4-d pretreatment period followed by a 4-d treatment period. During both periods, consumption was encouraged by food depriving the birds overnight.

During pretreatment periods, 24 birds were randomly assigned to six groups (n = 4 birds/group; 3 groups/test regime). Between 0900 and 1100, three groups of birds received one cup and three groups received two cups, each containing 10 g of ether-treated feed. Cups were positioned in the center front of each cage. At the end of the 2-h period, food cups were removed, and consumption was recorded. Birds were then given free access to maintenance feed until lights out.

During the treatment period, one one-cup and one two-cup test group were randomly assigned to each of the three chemical concentrations. During the 2-h test period, one-cup groups were given a single cup containing 10 g of treated feed. Two-cup groups were given two cups, one containing 10 g of treated feed, and the other containing 10 g of control feed (treated with ether only, as in pretreatment). To prevent birds from associating chemical treatments with cup position (left or right) or individual cups, the positions of the cups were alternated and different cups were presented each day. At the end of the test period, food cups were removed and consumption was measured. Birds were left with free access to maintenance feed until lights out.

Statistical analysis

For two-cup tests, the mean consumption of treated and control feed by each bird during each experiment was calculated. Means were examined in a three-factor analysis of variance (ANOVA) with repeated measures between cups. The independent factors were chemical and concentration. In addition, preference ratios were calculated by dividing consumption of treated feed by total consumption (treatment and control). Ratios were examined in a two-factor (chemical, concentration) ANOVA.

For one-cup tests, mean pretreatment and treatment consumption by each bird in each experiment was calculated. Means were examined in a three-factor ANOVA with repeated measures between periods. The independent factors in this analysis were chemical and concentration.

In all cases, Tukey's Honestly Significant Difference (HSD) tests were used to isolate significant (P < .05) differences among means (Winer 1962:198). Means are presented ± 1 SE.

RESULTS

Two-cup tests

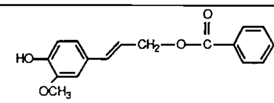
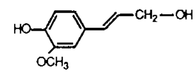
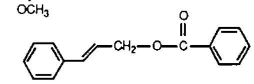
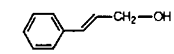
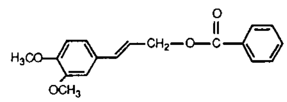
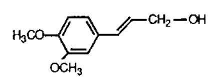
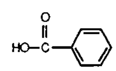
Overall, birds ate more control (3.34 ± 0.44 g) than treated feed (1.25 ± 0.62) (F = 244.8; 1, 62 df; P < .00001). This difference was concentration dependent, as indicated by post-hoc tests on the significant interaction (F = 18.6; 2,62 df; P < .00001) between concentration and cups.

There were also differences among chemicals. Post-hoc examination of the interaction between chemicals and cups (F = 6.4; 6,62 df; P < .0001) showed that birds given coniferyl alcohol, 3,4-dimethoxycinnamyl alcohol, or 3,4-dimethoxycinnamyl benzoate ate less treated feed (0.6 ± 0.3, 0.7 ± 0.2, 0.9 ± 0.3 g, respectively) than did birds given cinnamyl alcohol, cinnamyl benzoate, or coniferyl benzoate (1.3 ± 0.2, 1.4 ± 0.2, 1.4 ± 0.2 g, respectively). However, birds given benzoic acid ate more treated feed (2.1 ± 0.3 g) than any other group (Fig. 1). Consumption of control feed among groups was inverse to the consumption of treated feed.

Finally, post-hoc examination of the interaction among chemicals, concentrations, and cups (F = 2.03; 12,62 df; P < .03) revealed the following pattern of significant effects. For benzoic acid, there were no significant differences between consumption of treated and control feeds at any but the highest concentration. For cinnamyl benzoate, there was no difference in consumption at the lowest chemical concentration, but significant differences at medium and high concentrations. For cinnamyl alcohol, coniferyl benzoate, and 3,4-dimethoxycinnamyl benzoate, differences in consumption between treated and control feed increased with increasing concentrations. For 3,4-dimethoxycinnamyl alcohol, there were no differences in consumption at the lowest concentration, but consumption of treated feed was essentially eliminated at the medium and high concentration. For coniferyl alcohol, consumption of treated feed was strongly suppressed at all concentrations.

Analysis of preference ratios gave a pattern of results similar to those described above (Fig. 2). First, there were significant differences among chemicals (F = 9.7; 6,62 df; P < .00001). Post-hoc tests showed that the lowest preference ratios were associated with coniferyl alcohol (0.13), 3,4-dimethoxycinnamyl alcohol (0.19), and 3,4-dimethoxycinnamyl benzoate (0.20). Intermediate ratios were associated with cinnamyl alcohol (0.27), cinnamyl benzoate (0.32), and coniferyl benzoate (0.33). The highest mean ratio was associated

TABLE 1. Chemical structure and concentration (percent mass) of coniferyl and cinnamyl derivatives tested for inhibition of feeding by European Starlings in one- and two-cup tests. Concentrations are equimolar among chemicals (113, 56, and 14 μmol/g).

Compound	Structure	Concentrations tested (%)
Coniferyl benzoate		3.20, 1.60, 0.40
Coniferyl alcohol		2.03, 1.01, 0.25
Cinnamyl benzoate		2.68, 1.34, 0.66
Cinnamyl alcohol		1.52, 0.76, 0.19
3,4-dimethoxycinnamyl benzoate		3.36, 1.68, 0.42
3,4-dimethoxycinnamyl alcohol		2.19, 1.09, 0.27
Benzoic acid		1.38, 0.68, 0.17

to the birds. A double blind design was followed when preparing the diets to prevent possible measurement biases when measuring feed consumption.

Dietary concentrations for all compounds were equimolar with three concentrations of coniferyl benzoate (113, 56, and 14 μmol/g) (Table 1). These concentrations bracket the apparent avoidance threshold of Ruffed Grouse (*Bonasa umbellus*) for dietary coniferyl benzoate (Jakubas et al. 1989, Jakubas and Gullion 1990). Control diets were prepared by mixing the test feed with ethyl ether and evaporating the ether as described above.

Coniferyl alcohol is reportedly sensitive to light and will decompose slowly upon standing (Allen and Byers 1949; Aldrich Chemical Company, Milwaukee, Wisconsin). Therefore, its stability in feed was tested prior to the feeding trials. Four test samples were prepared by dissolving coniferyl alcohol (4 × 50 mg) (Aldrich Chemical Company, Milwaukee, Wisconsin) in ether and applying it to the bird's test feed (4 × 2.4 g). Following evaporation of the ether, the samples were stored under standard room temperature and light conditions for 2-, 4-, and 24-h periods. The fourth sample was stored in a closed container for 4 d at −17°C. Samples were extracted with ether and analyzed by thin-layer chromatography (TLC) for changes in coniferyl alcohol content. No significant change in coniferyl alcohol concentrations occurred in the −17°C sample or in the samples held under standard conditions for up to 24 h.

A comparison was made between the efficacy of pure coniferyl benzoate and Siam benzoin tears (a resin from *Styrax tonkinensis* [Adamson 1972]) as feeding repellents. Siam benzoin tears (or gum benzoin Siam) contain coniferyl benzoate, vanillin, benzoic acids, and cinnamic acids (Windholtz et al. 1983). Analysis by high-pressure liquid chromatography (HPLC) (Jakubas et al. 1989, Jakubas and Gullion 1990) indicated that Siam benzoin tears number 3 (Alfred L. Wolff, Paris, France) contained 80% coniferyl benzoate. Cinnamyl benzoate was not found in our benzoin Siam tears contrary to reports in Furia and Bellanca (1975). Benzoin Siam tears were dissolved in ether, filtered, and applied to the test feed in a manner identical to the application of other test compounds. Application levels of benzoin Siam tears were based on its coniferyl benzoate content and were matched to the test concentrations used for pure coniferyl benzoate (113, 56, and 14 μmol/g).

Feeding trials

European Starlings (*Sturnus vulgaris*) were funnel trapped in rural New Jersey and Philadelphia, Pennsylvania, from January to March, and transported to the laboratory. This species was used because it has good chemosensory abilities (Clark and Mason 1987, Mason et al. 1989, Espaillat and Mason 1990), and because it is considered an agricultural pest (Glahn et al. 1989).

Birds were individually caged (dimensions: 61 × 36 × 41 cm) and kept in constant temperature conditions (≈20°C), under an 11:13 h light:dark cycle. During

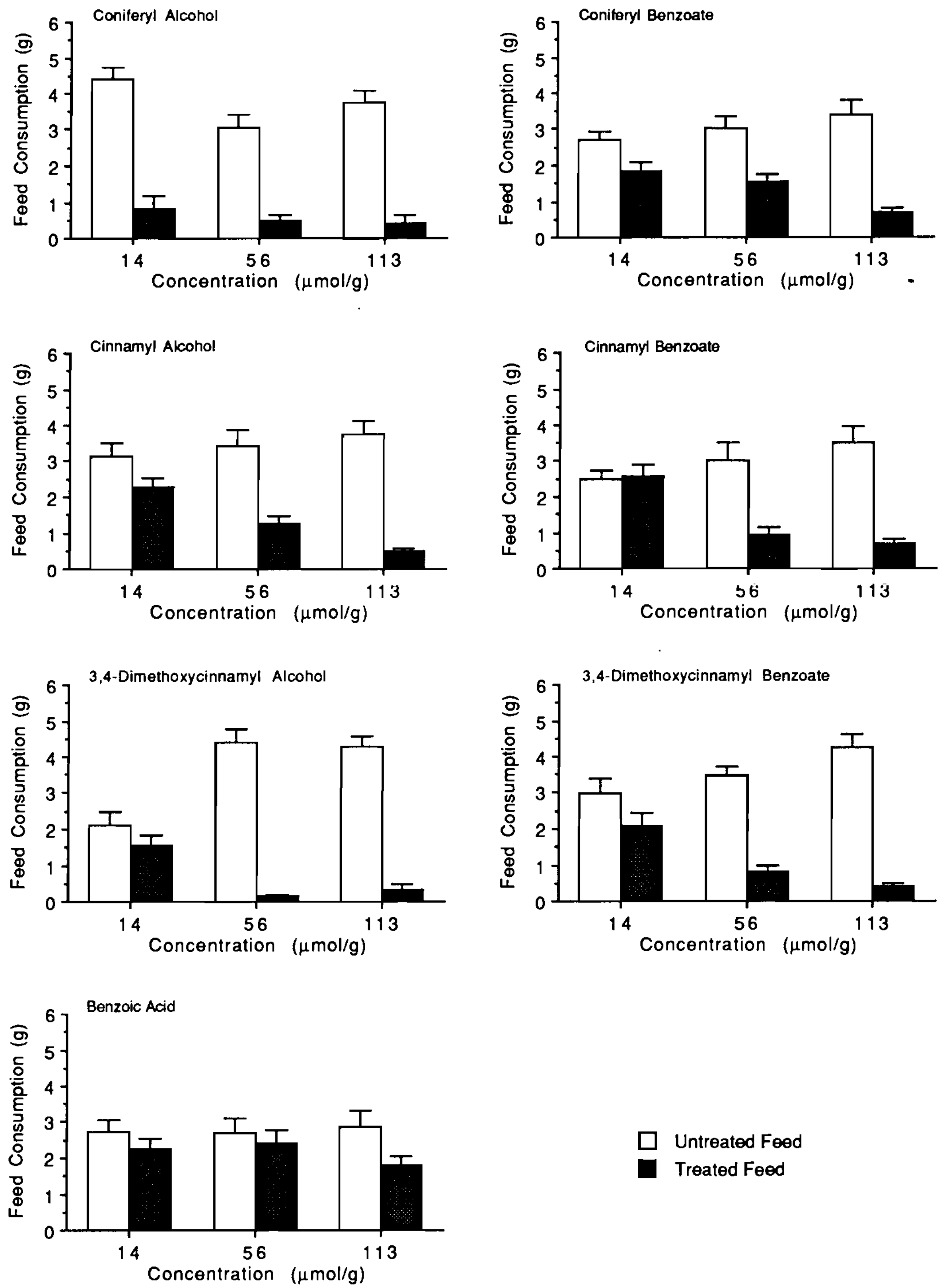


FIG. 1. Consumption of treated and control feed by starlings in two-cup (choice) tests. Data show means and 1 SE of the means.

with benzoic acid (0.44). Second, there was an inverse relationship between concentration and preference ratio size ($F = 29.4$; 2,62 df; $P < .00001$), i.e., the higher the concentration, the lower the ratio. Finally, there was a significant interaction between chemical and concentration ($F = 2.1$; 12,62 df; $P < .03$) (Fig. 2). Post-hoc tests indicated that preference ratios for all coniferyl alcohol concentrations were very low. Preference ratios for the lowest concentration of 3,4-dimethoxycinnamyl alcohol or 3,4-dimethoxycinnamyl benzoate

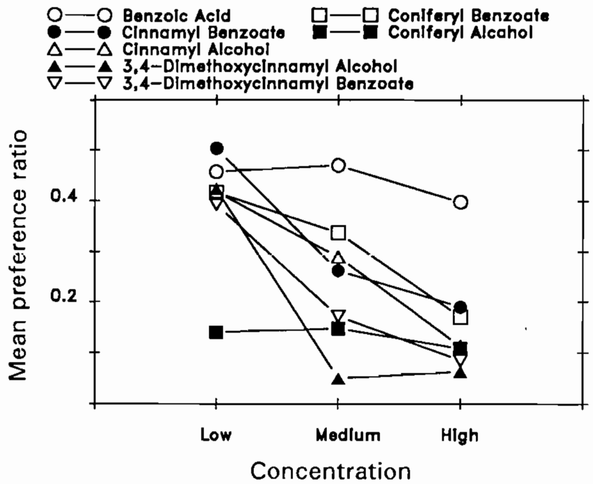


FIG. 2. Mean two-cup preference ratios, calculated by dividing treated feed consumption by total (treated plus control) consumption. A preference ratio of 1.0 indicates absolute preference for treated feed, a ratio of 0.0 indicates absolute rejection of treated feed, and a ratio of 0.5 indicates indifference between treated and control feed.

were higher than ratios for intermediate and high concentrations. Preference ratios for the lowest concentration of cinnamyl alcohol, cinnamyl benzoate, or coniferyl benzoate were higher than ratios for intermediate or high concentrations. However, the intermediate and high concentration ratios for these compounds were greater than ratios associated with intermediate or high concentrations of coniferyl alcohol, 3,4-dimethoxycinnamyl alcohol, or 3,4-dimethoxycinnamyl benzoate. Finally, preference ratios for any concentration of benzoic acid were high, and approached indifference (0.50).

One-cup tests

There were significant differences between periods and among concentrations. Not surprisingly, birds ate more during pretreatment than during treatment (F = 622.7; 1,70 df; P < .0001), and showed greater consumption at low concentrations than at intermediate or high concentrations (F = 33.5; 2,70 df; P < .0001). There were significant differences among chemicals (F = 10.8; 7,70 df; P < .0001), and post-hoc tests showed that overall (pretreatment and treatment) consumption was lowest for 3,4-dimethoxycinnamyl alcohol (2.9 ± 0.3 g) and coniferyl alcohol (3.2 ± 0.2 g). The next lowest consumption was exhibited by birds given 3,4-dimethoxycinnamyl benzoate (3.5 ± 0.3 g) or coniferyl benzoate (3.7 ± 0.2 g). Birds given cinnamyl benzoate or cinnamyl alcohol showed still higher consumption (4.0 ± 0.3, 4.1 ± 0.2 g, respectively). However, the highest overall consumption was exhibited by birds given either benzoin Siam (4.5 ± 0.3) or benzoic acid (4.5 ± 0.4 g).

Post-hoc evaluation of the interaction between chemicals and periods (F = 18.6; 7,70 df; P < .0001) showed that all chemicals except coniferyl benzoate significantly reduced consumption during the treatment period. Examination of the interaction between concentrations and periods (F = 116.78; 2,70 df; P < .0001) indicated that differences in consumption between periods became greater as concentrations increased. Finally, an assessment of the interaction among chemicals, concentrations, and periods (F = 4.0; 14,70 df; P < .0001) revealed a complex pattern (Fig. 3). At the lowest concentration, consumption differences between pretreatment and treatment periods were greatest for 3,4-dimethoxycinnamyl benzoate and cinnamyl benzoate. Consumption differences for 3,4-dimethoxycinnamyl benzoate were significantly > cinnamyl alcohol and benzoin Siam, while benzoin Siam, in turn, was > coniferyl alcohol, coniferyl benzoate, benzoic acid, and 3,4-dimethoxycinnamyl alcohol. Coniferyl benzoate, 3,4-dimethoxycinnamyl alcohol, and benzoic acid produced no drop in consumption at the lowest concentration; rather, consumption of treated feed was slightly higher than consumption of untreated feed for these compounds. At the intermediate concentration, 3,4-dimethoxycinnamyl benzoate, cinnamyl benzoate, 3,4-dimethoxycinnamyl alcohol, and benzoin Siam > coniferyl alcohol and cinnamyl alcohol, while coniferyl alcohol was > coniferyl benzoate and benzoic acid. At high concentration, benzoin Siam, cinnamyl benzoate, 3,4-dimethoxycinnamyl benzoate, and 3,4-dimethoxycinnamyl alcohol were similar in repellency. 3,4-dimethoxycinnamyl benzoate was significantly > benzoic acid and cinnamyl alcohol, while cinnamyl alcohol > coniferyl benzoate and coniferyl alcohol.

A comparison of the preference ratios for benzoin Siam tears and coniferyl benzoate indicates that benzoin Siam tears were significantly (F = 23.04; df 1,17; P = .0003) more repellent than pure coniferyl benzoate.

DISCUSSION

The results of the present studies were partly dependent upon experimental paradigm and chemical concentration. Overall, the results of two-cup tests suggested that coniferyl alcohol and 3,4-dimethoxycinnamyl alcohol were slightly more repellent than 3,4-dimethoxycinnamyl benzoate and cinnamyl alcohol. In turn, these latter compounds were more repellent than cinnamyl benzoate and coniferyl benzoate. Benzoic acid was only weakly aversive. Relative repellency among chemicals varied with concentration. Differences in repellency among chemicals were attenuated at low and high concentrations, except for coniferyl alcohol and benzoic acid. Coniferyl alcohol was significantly more repellent than other chemicals at low concentration, while benzoic acid was significantly less repellent than all other chemicals at high concentration.

The overall difference in consumption between one-cup treatment and pretreatment periods suggested that

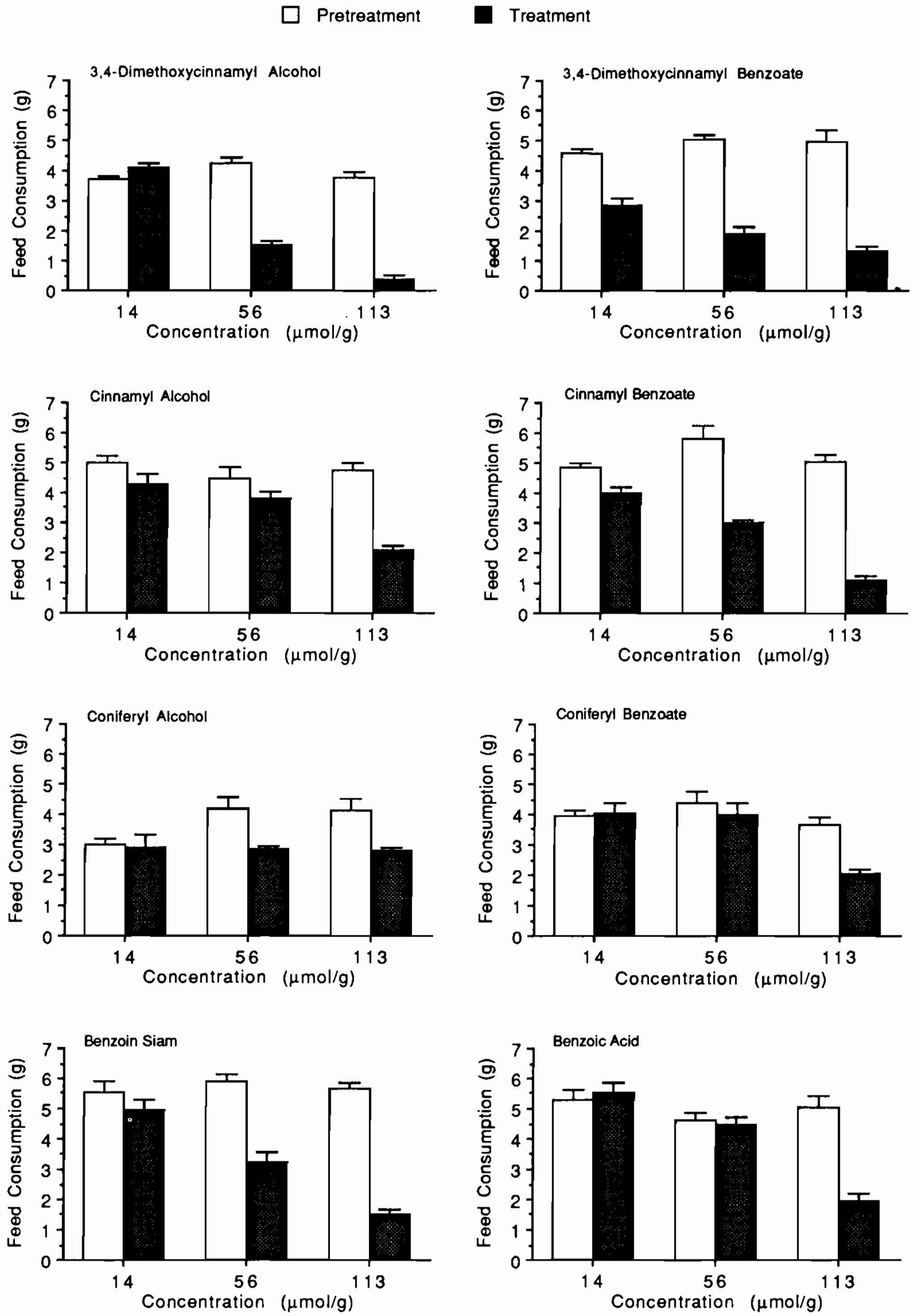


FIG. 3. Pretreatment and treatment consumption by starlings in one-cup (no-choice) tests. Data show means and 1 SE of the means.

3,4-dimethoxycinnamyl benzoate was the most repellent substance, followed by cinnamyl benzoate, and benzoin Siam. 3,4-dimethoxycinnamyl alcohol was more aversive than cinnamyl alcohol and benzoic acid, and cinnamyl alcohol was more repellent than coniferyl alcohol or coniferyl benzoate. These final two chemicals and benzoic acid were either weakly repellent (coniferyl alcohol and coniferyl benzoate) relative to other substances, or offensive only at high concentrations (benzoic acid). Over the 4-d treatment period, the starlings appeared to habituate to coniferyl benzoate and benzoin Siam treated feed (all concentrations), thus lowering the overall repellency of these compounds (see Jakubas and Mason 1991). As in the two-cup tests, relative repellency among chemicals varied with concentration. The least variation in repellency among chemicals occurred at low concentrations. At the low concentration, only 3,4-dimethoxycinnamyl benzoate was moderately repellent while other chemicals showed little or no repellency. As chemical concentrations increased benzoin Siam, 3,4-dimethoxycinnamyl alcohol, and benzoic acid increased in repellency relative to other chemicals, while coniferyl alcohol and cinnamyl alcohol decreased in relative repellency.

Two-cup tests are comparatively more sensitive than one-cup tests for detection of avoidance (Dragoin et al. 1971); therefore, we argue that one-cup tests provide a more rigorous measure of repellency in an absolute sense. For example, in one-cup tests, animals may be presented with the choice of eating unpalatable food or going hungry; however, in two-cup tests, the equivalent choice would be between palatable and unpalatable foods. Consequently, repellency can be harder to detect in one-cup tests as compared to two-cup tests. For this reason, our speculations on structure–activity relationships are developed from one-cup data. Although relative repellency was affected by concentration, the overall pattern of repellency among chemicals (especially at medium and high chemical concentrations) was fairly consistent. Three conclusions were inferred from these data. First, benzoate esters, in general, are more repellent than their corresponding alcohols. Second, repellency is increased by electron-donating groups (e.g., methoxy groups). Third, acidic functions (e.g., acids [-COOH] or phenol groups [-OH]) decrease repellency. The last conclusion is consistent with an earlier study that used feral pigeons to test the repellency of cinnamic acids. In that study, 3,4-dimethoxycinnamic acid and cinnamic acid were inoffensive in one-cup tests (Crocker and Perry 1990); however, we found that the alcohols and/or benzoate esters of these acids were repellent to starlings, at approximately the same concentration tested by Crocker and Perry.

Conversely, our speculations on structure–activity relations would differ on the role of acidic functions and benzoate esters, if our conclusions were based on the two-cup tests. This is principally due to the greater repellency of the alcohols, particularly coniferyl alcohol in these tests. The high repellency of coniferyl alcohol in two-cup tests contrasts with the lower repellency of other compounds in our study with acidic functions (benzoic acid and coniferyl benzoate), and similar compounds tested by Crocker and Perry (1990) (cinnamic acid and 3,4-dimethoxycinnamic acid). Determination of the properties of coniferyl alcohol that make it an effective repellent in two-cup tests would help clarify the structure–activity relationships observed in our study.

It is interesting that the repellency of alcohols relative to their corresponding esters were essentially reversed in one- and two-cup tests. Our data do not tease apart the roles played by the chemical senses (i.e., taste, olfaction, and trigeminal chemoreception) or postingestional effects (e.g., gastrointestinal malaise). However, we speculate that the alcohols may have had more offensive sensory properties, due to their greater volatility. Conversely, benzoate esters, although less offensive as chemical stimuli, may be more effective in inducing gastrointestinal malaise, especially when the birds were food deprived overnight. This result is suggested by the relatively greater repellency of benzoic acid, at high concentrations, in one-cup tests as compared to two-cup tests (preference ratios 0.28 and 0.40, respectively). The greater consumption of benzoic acid treated feed in the two-cup tests may have been due to the irritating properties of benzoic acid being buffered by the concurrent consumption of untreated feed. Similarly, in one-cup tests benzoic acid was not aversive unless presented at a high concentration.

Benzoin Siam, a resin from *Styrax tonkinensis* (Adamson 1972), which is primarily composed of coniferyl benzoate, was significantly more repellent than pure coniferyl benzoate at medium and high concentrations. Even at twice the concentration, coniferyl benzoate (3.2%) showed less repellency in one-cup tests than benzoin Siam (1.6% coniferyl benzoate), i.e., preference ratios 0.362 and 0.345, respectively. One explanation for the higher repellency of benzoin Siam is that other phenolic compounds in the resin may interact with coniferyl benzoate (e.g., synergistic or additive interactions) to enhance its repellent properties. Conversely, the higher repellency of benzoin Siam may be due to the inherent repellency of its other chemical constituents. Other phenolic compounds in benzoin Siam may enhance the repellency of coniferyl benzoate by increasing the severity of postingestional effects (e.g., Jung and Fahey 1983, Lindroth et al. 1988) or by intensifying the sensory qualities of coniferyl benzoate (see Jakubas and Mason 1991) through the stimulation of a greater number or variety of sensory receptors (e.g., Cowles et al. 1990).

Genetically engineering various agricultural crops to produce analogs of coniferyl alcohol may be feasible due to coniferyl alcohol's widespread occurrence in higher plants as the primary precursor of lignin. Many of the enzymes involved in the biosynthesis of coniferyl alcohol and related compounds have already been identified (Hahlbrock and Scheel 1989, Lewis and Yamamoto 1990). In addition, it may be possible to localize production of these compounds to specific plant tissues. For example, in quaking aspen coniferyl benzoate only occurs in the flower bud scales (Jakubas et al. 1989), and in wheat and oats, cinnamic acid related compounds predominantly occur in the bran of the grain (i.e., husks, pericarp, and aleurone) rather than in the more nutritious endosperm (Collins 1986, McCallum and Walker 1990). By localizing the production of repellent phenylpropanoids to specific plant tissues, autotoxic effects (if any exist) could be minimized along with the impact of these compounds on the nutritional value and palatability of the grain.

Compounds related to cinnamyl alcohol are, in general, believed to be relatively nontoxic (Hoskins 1984). Cinnamyl alcohol and cinnamyl benzoate have been cleared for human use (e.g., flavorings) by the U.S. Food and Drug Administration (Hoskins 1984). However, coniferyl alcohol and coniferyl benzoate have not been tested for their oral toxicity. The metabolism of phenolic esters and alcohols is, to a large extent, the same as that of their corresponding acid (Scheline 1978). Therefore, the metabolism of coniferyl alcohol and coniferyl benzoate should be similar to that of ferulic acid. In addition, ferulic acid would likely be one of the metabolic products resulting from the metabolism of 3,4-dimethoxycinnamyl alcohol (see Solheim and Scheline 1976). The toxicity of ferulic acid has not been fully reviewed; however, the minimum lethal dose that caused any mortality in a test animal (LD_{LO}) was relatively high (mouse LD_{LO} 1200 mg/kg) (Sweet 1987). However, when ferulic acid or its conjugated form feruloyl glucuronide were metabolized by rat intestinal microflora, 4-vinylguaiacol (4-hydroxy-3-methoxystyrene) was produced (Scheline 1968, 1978). Dietary 4-vinylguaiacol and ferulic acid reportedly interfere with the reproductive functions of female *Microtus montanus* (Berger et al. 1977). In addition, dietary ferulic acid affects the mating behavior of male Japanese Quail (*Coturnix coturnix*) (deMan and Peeke 1982). It should be kept in mind that the metabolism and toxicity of a given compound can vary greatly among animal species. The adverse reproductive effects of 4-vinylguaiacol may be limited to *Microtus montanus*; however, exposure to other styrenes is believed to adversely affect female human reproductive function (Nisbet and Karch 1983, Dixon 1986). Further toxicological tests on ferulic acid and related compounds appear warranted.

In addition to the structure–activity information presented here, an indirect result of this study was the identification of a class of naturally occurring compounds that are repellent to birds. The synthesis of compounds related to coniferyl alcohol in both plants and insects (see Keen and Littlefield 1979, Jakubas et al. 1989, Nishida and Fukami 1990) may represent another example of parallel production of repellents (see Rodriguez and Levin 1976). Although not all the physiological roles of these compounds are fully understood at this time, their repellent properties suggest that they may have a role in nature as defensive chemicals.

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Literature Cited

Adamson, A. D. 1972. Oleoresins production and markets with particular reference to the United Kingdom. Report of the Tropical Products Institute, Series **G 56**:1–46.

Allen, C. F. H., and J. R. Byers, Jr. 1949. A synthesis of coniferyl alcohol and coniferyl benzoate. Journal of the American Chemical Society **71**:2683–2684.

Berger, J. P., E. H. Sanders, P. D. Gardner, and N. C. Negus. 1977. Phenolic plant compounds functioning as reproductive inhibitors in *Microtus montanus*. Science **195**:575–577.

Bruggers, R. L., M. M. Jaeger, J. O. Keith, P. L. Hegdal, J. B. Bourassa, A. A. Latigo, and J. N. Gillis. 1989. Impact of fenthion on nontarget birds during quelea control in Kenya. Wildlife Society Bulletin **17**:149–160.

Bullard, R. W., and J. O. York. 1985. Breeding for bird resistance in sorghum and maize. Pages 193–222 *in* G. E. Russell, editor. Progress in plant breeding. 1. Butterworths, London, England.

Cardellina, J. H., II. 1988. Natural products in the search for new agrochemicals. Pages 305–315 *in* H. G. Cutler, editor. Biologically active natural products: potential use in agriculture. American Chemical Society, Washington, D.C., USA.

Clark, L., and J. R. Mason. 1987. Olfactory discrimination of plant volatiles by the European starling. Animal Behavior **35**:227–235.

Collins, F. W. 1986. Oat phenolics: structure, occurrence, and function. Pages 227–295 *in* F. H. Webster, editor. Oats chemistry and technology. American Association of Cereal Chemists, St. Paul, Minnesota, USA.

Cowles, R. S., J. R. Miller, R. M. Hollingworth, M. T. Abdel-All, F. Szurdoki, K. Bauer, and G. Matolcsy. 1990. Cinnamyl derivatives and monoterpenoids as nonspecific ovipositional deterrents of the onion fly. Journal of Chemical Ecology **16**:2401–2428.

Crocker, D. R., and S. M. Perry. 1990. Plant chemistry and bird repellents. Ibis **132**:300–308.

Cutler, H. G. 1988. Natural products and their potential in agriculture: a personal overview. Pages 1–22 *in* H. G. Cutler, editor. Biologically active natural products: potential use in agriculture. American Chemical Society, Washington, D.C., USA.

deMan, E., and H. V. S. Peeke. 1982. Dietary ferulic acid, biochanin A, and the inhibition of reproductive behavior in Japanese Quail (*Coturnix coturnix*). Pharmacology Biochemistry and Behavior **17**:405–411.

Dixon, R. L. 1986. Toxic responses of the reproductive system. Pages 432–477 *in* C. D. Klaassen, M. O. Amdur, and J. Doull, editors. Casarett and Doull's toxicology, the basic science of poisons. Macmillan, New York, New York, USA.

Dolbeer, R. A. 1988. Current status and potential of lethal means of reducing bird damage in agriculture. Pages 474–481 *in* H. Ouellet, editor. Acta Internationalis Ornithologici. Ottawa, Canada 1986. University of Ottawa Press, Ottawa, Canada.

Dragoin, W., G. E. McCleary, and P. McCleary. 1971. A comparison of measuring conditioned taste aversions. Behavior Research Methods and Instrumentation **3**:309–310.

Espaillat, J. E., and J. R. Mason. 1990. Differences in taste preference between Red-winged Blackbirds (*Agelaius* *phoeniceus*) and European Starlings (*Sturnus vulgaris*). Wilson Bulletin **102**:292–299.

Furia, T. E., and N. Bellanca, editors. 1975. Fenaroli's handbook of flavor ingredients. Second edition. Volume II. CRC, Cleveland, Ohio, USA.

Glahn, J. F., J. R. Mason, and D. R. Woods. 1989. Effects of dimethyl anthranilate as a bird repellent livestock feed additive. Wildlife Society Bulletin **17**:313–320.

Grote, F. W., Jr., and R. T. Brown. 1971. Conditioned taste aversions: two-stimulus tests are more sensitive than one-stimulus tests. Behavior Research Methods and Instrumentation **3**:311–312.

Hahlbrock, K., and D. Scheel. 1989. Physiology and molecular biology of phenylpropanoid metabolism. Annual Review of Plant Physiology and Plant Molecular Biology **40**:347–369.

Hoskins, J. A. 1984. The occurrence, metabolism and toxicity of cinnamic acid and related compounds. Journal of Applied Toxicology **4**:283–292.

Jakubas, W. J., and G. W. Gullion. 1990. Coniferyl benzoate in quaking aspen: a ruffed grouse feeding deterrent. Journal of Chemical Ecology **16**:1077–1087.

Jakubas, W. J., and G. W. Gullion. 1991. Use of quaking aspen flower buds by Ruffed Grouse: its relationship to grouse densities and bud chemical composition. Condor **93**:473–485.

Jakubas, W. J., G. W. Gullion, and T. P. Clausen. 1989. Ruffed grouse feeding behavior and its relationship to the secondary metabolites of quaking aspen flower buds. Journal of Chemical Ecology **15**:1899–1917.

Jakubas, W. J., and J. R. Mason. 1991. Role of avian trigeminal sensory system in detecting coniferyl benzoate, a plant allelochemical. Journal of Chemical Ecology **17**:2213–2221.

Jung, H. G., and G. C. Fahey, Jr. 1983. Effects of phenolic monomers on rat performance and metabolism. Journal of Nutrition **113**:546–556.

Keen, N. T., and L. J. Littlefield. 1979. The possible association of phytoalexins with resistance gene expression in flax to *Melampsora lini*. Physiological Plant Pathology **14**:265–280.

Kuroyanagi, M., K. Fujita, M. Kazaoka, S. Matsumoto, A. Ueno, S. Fukushima, and M. Katsuoka. 1985. Studies on the constituents of *Baccharis genistelloides*. Chemical and Pharmaceutical Bulletin **33**:5075–5078.

Lamb, C. J., M. A. Lawton, M. D. Dron, and R. A. Dixon. 1989. Signals and transduction mechanisms for activation of plant defense against microbial attack. Cell **56**:215–224.

Lewis, N. G., and E. Yamamoto. 1990. Lignin: occurrence, biogenesis and biodegradation. Annual Review of Plant Physiology and Plant Molecular Biology **41**:455–496.

Lindeberg, O. 1980. Synthesis of coniferyl and dihydroconiferyl derivatives using radical bromination with *N*-bromosuccinimide as the key step. Acta Chemica Scandinavica **B34**:15–20.

Lindroth, R. L., J. M. Scriber, and M. T. S. Hsia. 1988. Chemical ecology of the tiger swallowtail: mediation of host use by phenolic glycosides. Ecology **69**:814–822.

Lynn, D. G., and M. Chang. 1990. Phenolic signals in cohabitation: implications for plant development. Plant Physiology and Plant Molecular Biology **41**:497–526.

Mason, J. R., M. A. Adams, and L. Clark. 1989. Anthranilate repellency to starlings: chemical correlates and sensory perception. Journal of Wildlife Management **53**:55–64.

McCallum, J. A., and J. R. Walker. 1990. Phenolic biosynthesis during grain development in wheat: changes in phenylalanine ammonia-lyase activity and soluble phenolic content. Journal of Cereal Science **11**:35–49.

Nisbet, I. C. T., and N. J. Karch. 1983. Chemical hazards to human reproduction. Noyes Data, Park Ridge, New Jersey, USA.

Nishida, R., and H. Fukami. 1990. Sequestration of distasteful compounds by some pharmacophagous insects. Journal of Chemical Ecology **16**:151–164.

Ponpipom, M. M., R. L. Bugianesi, D. R. Brooker, B. Z. Yue, S. B. Hwang, and T. Y. Shen. 1987. Structure–activity relationships of kadsurenone analogues. Journal of Medicinal Chemistry **30**:136–142.

Robinson, T. V. 1983. The organic constituents of higher plants. Fourth edition. Cordus, North Amherst, Massachusetts, USA.

Rodriguez, E., and D. A. Levin. 1976. Biochemical parallelisms of repellents and attractants in higher plants and arthropods. Pages 214–270 *in* J. W. Wallace and R. L. Mansell, editors. Recent advances in phytochemistry. Volume X. Biochemical interactions between plants and insects. Plenum, New York, New York, USA.

Scheline, R. R. 1968. Metabolism of phenolic acids by the rat intestinal microflora. Acta Pharmacologica et Toxicologica **26**:189–205.

———. 1978. Mammalian metabolism of plant xenobiotics. Academic Press, London, England.

Solheim, E., and R. R. Scheline. 1976. Metabolism of alkenebenzene derivatives in the rat. II. Eugenol and isoeugenol methyl ethers. Xenobiotica **6**:137–150.

Sweet, D. V., editor. 1987. Registry of toxic effects of chemical substances. 1985–1986 edition. United States Department of Health and Human Services, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Washington, D.C., USA, Publication **87-114**.

Windholtz, M., S. Budavari, R. F. Blumetti, and E. S. Otterbein, editors. 1983. The Merck index. Tenth edition. Merck, Rahway, New Jersey, USA.

Winer, B. J. 1962. Statistical principles in experimental design. McGraw-Hill, New York, New York, USA.